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## Selective replication of ribosomal DNA repeats after loss of the abnormal oocyte phenotype in *Drosophila melanogaster*

(Southern blotting technique/nontranscribed spacer)

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**ABSTRACT** *Drosophila melanogaster* females homozygous for the abnormal oocyte mutation produce a large excess of female offspring when crossed with  $\widehat{XY}/0$  males. After several generations in *abo* homozygous stock, this maternal effect is no longer observed. The disappearance of the *abo* phenotype is coupled with an increase in the amount of DNA coding for rRNA (rDNA). We have used restriction endonuclease analysis of total DNA extracted from adult females and from single female larval brains to investigate the molecular organization of rDNA before and after the loss of the *abo* phenotype. The rDNA increase is associated with variations of the restriction pattern of the nontranscribed spacer, probably due to a selective increase of rDNA repeats.

*Drosophila melanogaster*  $X/X$  females homozygous for the abnormal oocyte mutation (*abo* 2:38) (1) crossed to attached  $\widehat{XY}/0$  males with a wild-type second chromosome produce a large excess of female offspring (2) as a result of sex-specific embryonic lethality. This maternal effect can be decreased or abolished by increasing the dose of the heterochromatic  $X$  chromosome segment, and it can be reduced by lowering the temperature (1, 2). No other visible phenotype appears in either *abo* males or females or their offspring (3). These data led Parry and Sandler (3) to the hypothesis that *abo*<sup>+</sup> controls the function of element(s) in the distal portion of the heterochromatic region of the chromosome, called  $Xh^{abo}$ . This heterochromatic region could influence or include the structural cistrons for the rRNA (rDNA), which are located at the bobbed locus. Recent studies indicate that in *abo* homozygotes the *abo* effect, as measured by the sex ratio in crosses to attached  $\widehat{XY}$  males, gradually decreases so that after several generations, the maternal effect is no longer observed. During this process, the rDNA content of each  $X$  chromosome (about 0.15% of the haploid genome) increases about 3-fold (4, 5).

We have used *Hind*III and *Hae* III restriction endonuclease analysis of total DNA extracted from adult females and from single female larval brains to investigate the molecular organization of rDNA before and after the loss of the *abo* phenotype. Combined use of *Hind*III and *Hae* III leaves the nontranscribed spacer almost intact, while the transcribed region of rDNA and the two types of insertion are digested into smaller fragments (6, 7).

Hybridization of blotted fragments with <sup>32</sup>P-labeled nontranscribed spacer indicates that the loss of the *abo* phenotype and the increase in the rDNA is coupled with variations of the restriction pattern of nontranscribed spacer, due to a selective replication of certain rDNA repeats.

### MATERIALS AND METHODS

**Drosophila Stocks.**  $XY^L.Y^S$ ,  $ywf$  and  $C(1)RM$ ,  $yw$  was from the University of Chicago collection and carries no extra  $Y$  chromosome. Wild-type Canton S with the second chromosome *abo/Cy* was obtained from the University of Chicago;  $X/X$  *abo/abo* females were tested for *abo* phenotype by mating to  $XY^L.Y^S ywf/0$  males, abbreviated as  $\widehat{XY}/0$ . The male offspring were tested for the presence of an extra  $Y$  chromosome by measuring their fertility. An *abo* homozygous stock was constructed by mating  $X/X$ ; *abo/abo* females to  $X/Y$ ; *abo/abo* males.

**DNA Extraction.** The DNA from a population of flies was extracted as described (8). DNA from single brains was extracted according to Endow and Glover (9).

A cloned fragment of nontranscribed spacer inserted into plasmid pBR322 was obtained from P. K. Wellauer. This fragment had been obtained after *Hind*III/*Hae* III digestion of plasmid Dmra56 (10) and is constituted by the 4.0-kilobase (kb) entire nontranscribed spacer and a region of 900 base pairs that is transcribed into the external spacer (11). Cloned DNA was prepared as described in Dawid *et al.* (12). Nick-translations were performed according to Rigby *et al.* (13). Restriction endonuclease digestions were performed according to the manufacturer's instructions [Bethesda Research Laboratories (Rockville, MD), New England BioLabs, and Miles]. Some *Hind*III digestions were performed with enzyme kindly supplied by R. Di Lauro. DNA fragments were separated by gel electrophoresis on 0.8% agarose gel in Tris/borate/EDTA buffer with *Hind*III-digested  $\lambda$  DNA as marker. Blotting was according to Southern (14) on Schleicher & Schuell nitrocellulose filters. Preincubation and hybridization were according to Endow and Glover (9). Filters were exposed on flash-activated autoradiographic film (Kodak 5R), using an intensifying screen (15).

<sup>3</sup>H<sup>3</sup>-Labeled rRNA was prepared as described (8) and rRNA-DNA hybridization was according to Gillespie and Spiegelman (16).

### RESULTS

Homozygous *abo* (first generation,  $G_0$ ) and heterozygous females were crossed to  $\widehat{XY}/0$  males. Table 1 presents the results of these crosses. As the data illustrate, *abo* is a recessive allele. We performed the same crosses with *abo/abo* homozygous females after 19 generations in homozygous condition ( $G_{19}$ ). In this last cross a practically normal sex ratio—that is, the loss of the *abo* phenotype—was observed.

A fertility test of the male offspring of the  $G_{19}$  cross showed

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Abbreviations: rDNA, structural cistrons for rRNA; kb, kilobase(s).

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Table 1. rDNA content of *X/X* females with the indicated chromosome 2 constitution, expressing or not the *abo* phenotype when crossed with *XY<sup>L</sup>.Y<sup>S</sup>y w f/0* males

Genotype	Phenotype	Sex ratio in the progeny, ♂/♀	% rDNA
<i>abo/Cy</i>	Wild type	4587/4312 = 1.06	0.285 ± 0.023
<i>abo/abo</i> G <sub>0</sub>	Mutant	591/6328 = 0.09	0.290 ± 0.024
<i>abo/abo</i> G <sub>19</sub>	Wild type	2981/3428 = 0.89	0.658 ± 0.038
<i>abo/Cy</i>	Wild type	3150/3206 = 0.98	0.287 ± 0.018
<i>abo/abo</i> G <sub>0</sub>	Mutant	289/2601 = 0.09	0.300 ± 0.021

The last three hybridizations were performed with DNA extracted from females with an equal number of generations in the respective stocks. rDNA is expressed as percent of total DNA, mean ± SEM.

there is no accumulation of free Y chromosomes in the parental females. Table 1 also illustrates the rDNA content measured by hybridization of the labeled rRNA to DNA. DNA was extracted from adult females of the following genotype: *abo/Cy* heterozygous, *abo/abo* G<sub>0</sub> homozygous females, and *abo/abo* G<sub>19</sub> females that no longer showed the *abo* phenotype.

*abo/Cy* and *abo/abo* G<sub>0</sub> females have 0.29% rDNA. As expected, because *abo* is recessive, after these additional 19 generations in heterozygous condition, the rDNA content remains unchanged (Table 1; compare lines 1 and 2 with 4 and 5). On the contrary, a 2-fold increase in rDNA per X chromosome (0.65% rDNA) is observed in adult G<sub>19</sub> *abo/abo* females that had a normal sex ratio when crossed to *X̄Y/0* males. This last value, even though lower than previously reported (4, 5), confirms the increase of rDNA after the loss of the *abo* phenotype.

To investigate the molecular organization of the rDNA, total DNA extracted from *abo/Cy* adult females and from G<sub>0</sub> *abo/abo* females was analyzed by means of double digestion with *Hind*III and *Hae* III restriction endonucleases. Fragments were separated on agarose gels, then blotted and hybridized with labeled probes from cloned nontranscribed spacer. Results (Fig. 1, lanes a and b) show that there is a wide length heterogeneity of nontranscribed spacers of *abo/Cy* and *abo/abo* G<sub>0</sub> females, ranging between 15 and 2.5 kb, with discrete length classes, 4.5 kb being the predominant class; however the two types of female have the same pattern of length heterogeneity. Furthermore, the rDNA extracted from single female larval brain has exactly the same length pattern as the rDNA from the stock, thus indicating that the observed pattern is not the average of different individual patterns (Fig. 2, lanes a-e).

The restriction pattern of total DNA extracted from adult G<sub>19</sub> females is shown in Fig. 1, lane c. The lengths of nontranscribed spacers of these females range between 18 and 2.4 kb. In addition to the predominant class of 4.5 kb present in the original stock, new bands are observed. The most evident band has a length of 3.8 kb, but new bands of 18, 3.4, 3.2, and 2.8 kb, and smaller, are detectable. Experiments performed on DNA extracted from individual larval brains of the same *abo/abo* G<sub>19</sub> females shows (Fig. 2, lanes f-k) that in this case also the patterns of single fly brains correspond to those observed for DNA extracted from population samples.

The presence of classes of nontranscribed spacers overrepresented in the G<sub>19</sub> phenotypically reverted females suggests that differential replication of the rDNA genes occurs during this process and that, in the ribosomal gene cluster, only some classes of rDNA repeats are augmented with respect to the others.

## DISCUSSION

The main purpose of our study was to analyze the molecular organization of ribosomal genes before and after the loss of the

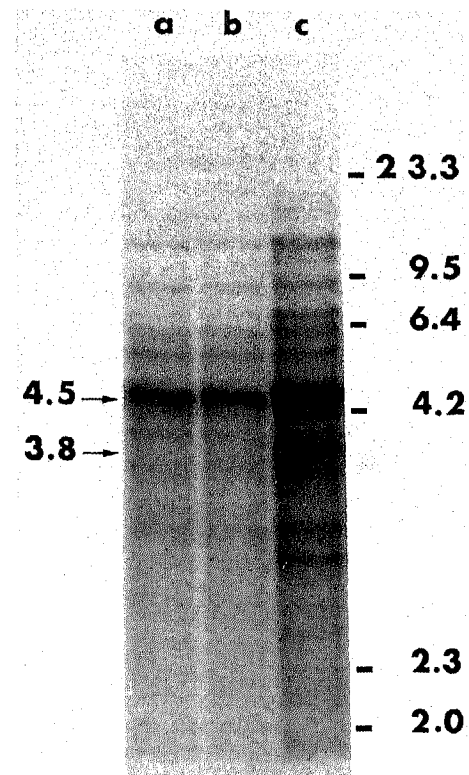


Fig. 1. *Hind*III/*Hae* III double digests of DNA extracted from populations of *abo/Cy* females (lane a), *abo/abo* G<sub>0</sub> females (lane b), and *abo/abo* G<sub>19</sub> females (lane c). Arrows indicate the band at 4.5 kb and the major new band at 3.8 kb.

*abo* phenotype by means of restriction analysis, using the nontranscribed spacer as a molecular marker of rDNA organization.

The results indicate that the patterns of nontranscribed spacers in heterozygous *abo/Cy* females and in first generation *abo/abo* females are identical. On the contrary, new fragments, especially one at 3.8 kb, are found in *Hind*III/*Hae* III double digests of DNA extracted from *abo/abo* females after 19 generations, when the *abo* phenotype is no longer observed. The same bands are also found in brains of single female larvae.

It is difficult to explain this appearance of a new pattern. The *abo/abo* females used to "found" the homozygous stock had the same pattern as that of the heterozygous females, and no variations have been found among individuals of the stocks. These observations rule out the possibility of strong selective pressure in favor of a particular rDNA organization. The results obtained with DNA extracted from the diploid and near-diploid tissue of single female larval brains indicate that the new pattern is not the average of different individual patterns and, in addition, that this is a general phenomenon rather than a particular event occurring in tissues with polytene chromosomes. Our data suggest that the new pattern may be a result of a selective increase of particular ribosomal genetic units present in undetectable amounts in first generation *abo/abo* females.

Other examples of selective amplification of particular rDNA repeats are known, even though only at a somatic level. In recent studies it was demonstrated that during polytenization (17) the genes from only one nucleolus organizer are replicated and that, among these genes, some repeats are preferentially replicated. This last observation implies that the entire ribosomal block is not uniformly replicated in polytene cells, suggesting that polytenization of rDNA may occur by an extrachromosomal mechanism.

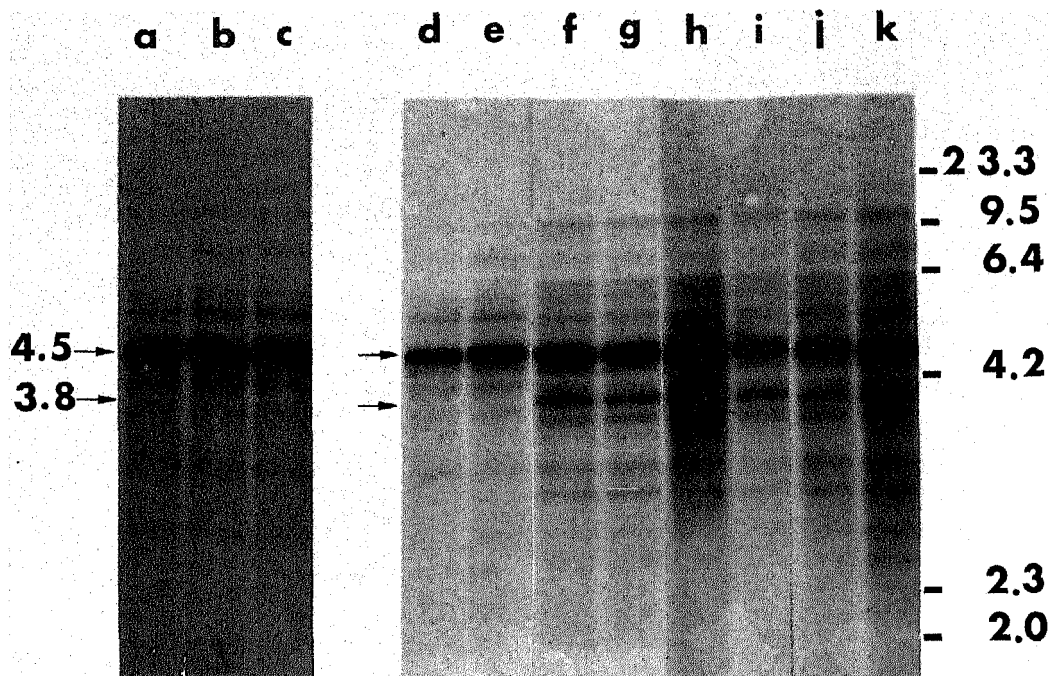


FIG. 2. *Hind*III/*Hae*III double digests of DNA extracted from *abo/abo* G<sub>0</sub> (lanes a–c), from *abo/Cy* (lanes d and e), and *abo/abo* G<sub>1,9</sub> (lanes f–k) single female larval brains. Lanes d–g were on the same gel. Arrows indicate the band at 4.5 kb and the major new band at 3.8 kb.

Because it appears that the selective rDNA increase observed during the reversion of *abo/abo* homozygous females is not necessary (18) for the suppression of the *abo* phenotype, this phenomenon could be concomitant with other more important events occurring in the heterochromatic region of the X chromosome.

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